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NOTE: This version supersedes all previous versions

Counting Lugols Preserved Phytoplankton Samples

Examination of preserved phytoplankton samples has some benefits over enumeration of live samples. Preserved samples do not have to be counted immediately. They can be settled for more accurate enumeration of cells and larger volumes can be examined. While the procedure below describes examination of preserved samples for enumeration of *Karenia* species, it can be used for other preserved dinoflagellates, flagellates, and diatoms.

Equipment:

- 5 mL polystyrene disposable standard serological pipets graduated in 1/10 mL (Fisher cat# S68228C**)**
  - **NOTE: DO NOT REUSE PIPETS**
- Pipet dispenser (Fisher cat# 13-681-102A or 13-681-102A)
- Lab-Tek™ 2-chambered Coverglass System (Fisher cat# 12-565-336; cat# 12-565-471 if the former is out of stock)
- Lugols Stock Solution (prepared in-house; See SEM staff)
- Inverted microscope with objective/ocular combination to magnify 100-200x
- Versa-Clean (Fisher cat# 04-342)
- Foam-Tipped Swabs (Fisher cat# 14-960-3J)
- Counter or multi-unit counter for counts of more than one species
- Data sheets to always include the following:
  HAB#, collection date, collection location, geographic coordinates, chamber number, volume settled, analysis date, analyst, genus and species, raw cell count numbers and calculated cells/liter concentrations.

Methods:

Previously preserved samples

1. Gently invert the sample 20 times (40 cycles). Remove the cap and immediately withdraw 3 mL of sample into the pipet. Dispense the sample in one of the two chambers of the NUNC system. Allow the sample to settle for **at least 30 minutes** before counting. If the sample is not counted right away, store the chamber in the dark, with the chamber lid on, and analyze within 48 hours. If the settled sample is not examined within 48 hours discard chamber and settle a fresh aliquot of material.

2. To enumerate species in low concentrations scan chamber at low magnification (~100x), moving row by row to cover the entire chamber for all genera. Scan the entire 3 mls to enumerate all species in low concentration and to determine diatom range. **NOTE**: **THIS IS A MANDATORY STEP.**

3. To obtain the *number of cells*/liter, when counting the 3 ml chamber, divide the number of cells counted in the entire chamber by 3, and multiply by 10^3

- Record significant digits only (to the 10^3) for *example:*
  667*;* 7600; 18,600; 1,857,000
- Record data to be recorded in **permanent ink** on datasheets **(THIS IS MANDATORY)**

4. For blooms of *Karenia brevis* or other toxic phytoplankton, *or Pseudo-nitzschia* spp. where high cell concentration inhibits accurate counting, examine a re*duced portion* of the chamber (from Step #2) by scanning 1-3 transects in the middle of the chamber at a higher magnification (~200x-400x). As an Example: the Olympus IX71 inverted microscope has 21 rows at 20x and 28 rows at 40x. If 2 transects are read at 20x and 7 *Karlodinium veneficum* cells are counted then 7 is divided by 2 to get a number of cells/transect. 3.5 is then multiplied by 21 to get the raw number of cells for the chamber).

5. To obtain the number of cells/L when a reduced portion of the chamber is counted, divide the number of cells counted by the number of transects that were counted. Next, multiply the total number of rows at that specific magnification. This yields the number of cells per chamber; divide by 3 and multiply by 10^3 to give cells/L.

   - Record significant digits only (to the 10^3) for *example:*
     667*;* 7600; 18,600; 1,857,000
   - Record data to be recorded in **permanent ink** on datasheets **(THIS IS MANDATORY)**

Live Samples

1. Gently invert the sample 20 times (40 cycles). Remove the cap and immediately withdraw 3 mL of sample into the pipet. Dispense the sample in one of the two chambers in the NUNC system. Add one drop of Lugols stock solution to the chamber well... Allow the sample to settle for at least 30 minutes before counting. If the sample is not counted right away, store the chamber in the dark with the chamber lid and analyze within 48 hours.
2. Follow Step #2 and then Step #3 (if necessary) from previous section.

Notes:
Lugol's preservative makes the surface of the cells sticky. They will stick to the sample bottles, the pipets and the chambers. To prevent sample cross contamination:

- Wash the sample bottles with a Versa-Clean solution between uses
- Clean the chambers the day of use. Do not soak. Clean them with a Versa-Clean solution and foam-tipped sponge. Rinse with deionized water after use. **DO NOT** use a chamber more than 3 times.